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SURVEY AND SUMMARY

Mammalian Base Excision Repair: the Forgotten Archangel

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ABSTRACT

Base excision repair (BER) is a frontline repair system that is responsible for maintaining genome integrity and thus preventing premature aging, cancer and many other human diseases by repairing thousands of DNA lesions and strand breaks continuously caused by endogenous and exogenous mutagens. This fundamental and essential function of BER not only necessitates tight control of the continuous availability of basic components for fast and accurate repair, but also requires temporal and spatial coordination of BER and cell cycle progression to prevent replication of damaged DNA. The major goal of this review is to critically examine controversial and newly emerging questions about mammalian BER pathways, mechanisms regulating BER capacity, BER responses to DNA damage and their links to checkpoint control of DNA replication.

BASE EXCISION REPAIR: BASIC FACTS

DNA lesions arise owing to the intrinsic chemical instability of the DNA molecule in the cellular milieu, which results in hydrolytic loss of DNA bases, base oxidations, non-enzymatic methylations and other chemical alterations, as well as because of multiple reactions with exogenous (environmental) and endogenous (intracellular) DNA reactive species (1,2). If left unrepaired, such DNA alterations may interfere with DNA replication and transcription, resulting in the accumulation of mutations and a disturbance in cellular metabolism. Among the many strategies to maintain a smooth operation and reproduction of the DNA blueprint, base excision repair (BER) is an essential repair pathway that corrects multiple DNA alterations that frequently occur in DNA. BER deficiency affects genome stability and is implicated

in many human diseases, including premature aging (3), neurodegeneration (4) and cancer (5). It is estimated that every single human cell has to repair 10 000–20 000 DNA lesions every day (1). Enzymes involved in BER recognize damaged DNA bases and catalyze excision of the damaged nucleotide and its replacement with a new undamaged one. The majority of BER is accomplished through the so-called short-patch BER and results in removal and replacement of only one nucleotide (6–8). Naturally, as nucleotide excision during BER leads to the transient formation of a DNA single-strand break (SSB), BER enzymes are also the major players in SSB repair (9). BER reactions in cells are extremely fast, and in many cases, an individual BER event may take only a few minutes (10,11). The repair of acute DNA damage requires several rounds of BER and can take several hours, as the amount of BER enzymes is limited.

BASE EXCISION REPAIR: MECHANISMS AND PATHWAYS

The major players involved in BER have been known for a long time (12) and the entire BER process has been reconstituted with purified enzymes (13,14). BER is initiated by a damage-specific DNA glycosylase that recognizes the damaged DNA base and cleaves the *N*-glycosylic bond that links the DNA base to the sugar phosphate backbone (15, Figure 1). Currently, 11 human DNA glycosylases that recognize and excise a wide range of DNA base damages are described (Supplementary Table S1). The arising baseless site (also called abasic site, apurinic/apyrimidinic site or AP site) is further processed by an AP endonuclease (APE1 in human cells) that cleaves the phosphodiester bond 5' to the AP site, thus generating a SSB, also called a nick, containing a hydroxyl residue at the 3'-end and deoxyribose phosphate at the 5'-end.

At this point, the repair of damaged DNA bases converges with SSB repair. To accomplish repair, the SSB must have 3'-hydroxyl and 5'-phosphate ends that will

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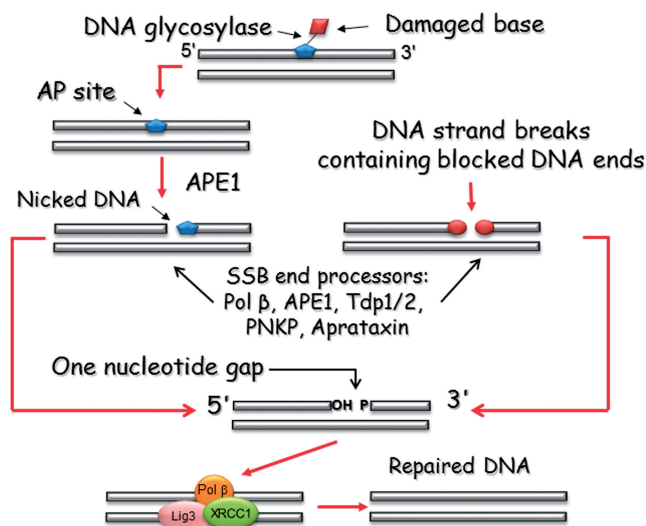


Figure 1. Simplified scheme for the major base excision repair pathway. ‘Blocked’ DNA strand breaks may arise as a result of direct chemical modification during SSB formation or during enzymatic processing of DNA base damage by a DNA glycosylase and AP-endonuclease. A SSB containing a one nucleotide gap with 3'-hydroxyl and 5'-deoxyribose phosphate ends is recognized by Pol β , which fills the gap, removes the 5'-deoxyribose phosphate and recruits XRCC1–DNA ligase III α complex to seal the DNA ends (‘classic’ BER pathway, left branch of the scheme). Strand breaks containing other DNA ends blocking modifications are recognized by the corresponding damage-specific protein that converts 5'- and/or 3'-ends into the conventional 5'-phosphate and 3'-hydroxyl ends and further recruits Pol β and XRCC1–DNA ligase III α to accomplish repair (right branch of the scheme). Among the known damage-specific protein are Pol β , APE1, PNKP, TDP1, TDP2 and aprataxin.

allow a DNA polymerase to incorporate a new nucleotide and DNA ligase to seal the DNA ends. In the ‘classic’ case of BER that is initiated by the so-called monofunctional DNA glycosylases, ligation of the SSB is prevented by the 5'-deoxyribose phosphate. Therefore, DNA polymerase β (Pol β) using its AP lyase activity removes this blocking group (16) and simultaneously adds one nucleotide to the 3'-end of the nick. To finalize DNA repair, the XRCC1–DNA ligase III α complex seals the DNA ends (17–19). Many other SSBs, arising endogenously or after mutagenic insults, similarly contain unligatable ends that need further processing. For example, repair of oxidative base lesions is frequently initiated by DNA glycosylases that have an associated β -lyase activity which, in addition to removing damaged DNA base, also cleaves the phosphodiester backbone 3' to the AP site to generate a nick with 3'- α,β -unsaturated aldehyde (20,21). Formation of blocking lesions is also apparent during BER conducted by the Neil DNA glycosylases, which, in addition to the DNA glycosylase activity, are also able to excise the arising AP site by β,δ -elimination, leaving a 3'-phosphate containing nick (22). DNA SSBs containing damaged 3'-ends may also arise as a result of direct damage to deoxyribose (23). Endogenous oxidative metabolism and exogenous factors, such as ionizing radiation generating reactive oxygen species, in addition to producing oxidative DNA base modifications and AP sites, can also directly induce SSBs with modified 5'- and/or 3'-ends (24). There

are also several other types of blocked SSBs generated by aborted activity of DNA ligases or by DNA topoisomerase I and II (25–27). Because the formation of non-canonical SSBs blocks further repair, a group of DNA damage-specific enzymes cleans up the SSB ends and thereby prepares them for DNA synthesis and ligation (Figure 1). The five known SSB end-processors are (i) Pol β , which removes blocking 5'-sugar phosphates (16); (ii) APE1 that removes 3'-sugar phosphates (28); (iii) Polynucleotide Kinase Phosphatase (PNKP) that dephosphorylates 3'-ends and phosphorylates 5'-hydroxyl ends (29); (iv) Aprataxin that cleans 5'-termini blocked by abortive ligation reactions (27) and (v) tyrosyl DNA phosphodiesterases TDP1 that repair SSBs generated by abortive DNA topoisomerase reactions (26,30). These end-processing enzymes, separately or in combination, can convert the SSB to a one-nucleotide gap with 3'-hydroxyl and 5'-phosphate ends that can be filled by Pol β and finally ligated by the XRCC1–DNA ligase III α complex (Figure 1).

If the 5'-ends are blocked and cannot be processed by the five SSB end-processing enzymes mentioned above, BER can be accomplished by the long-patch sub-pathway (31–33). This pathway is also initiated by Pol β -dependent incorporation of the first nucleotide into the nick and is continued by enzymes borrowed from the lagging strand replication machinery (34,35). The replicative Pol δ continues strand displacement synthesis in the presence of proliferating cell nuclear antigen and replication factor C. The resulting flap of 2–12 nucleotides is cut off by flap endonuclease 1 and the final nick sealed by DNA ligase I (36).

BASE EXCISION REPAIR IS THE FOUNDATION OF GENOME STABILITY

Although there is no convincing evidence for cell cycle regulation of BER, based on the biochemical properties of BER enzymes, the majority of which prefer double-stranded DNA substrates, it is reasonable to assume that BER mainly operates through the G1 phase of the cell cycle. During G1, BER activity maintains error-free transcription and prepares DNA for replication by removing DNA lesions. However, if DNA base damage is not removed before the initiation of DNA replication, genome integrity is assured by a backup system called translesion DNA synthesis (TLS) that involves specialized Pols, which can perform error-free DNA synthesis over a wide range of DNA base lesions (Figure 2). Human cells possess 15 Pols, 11 of which are TLS Pols and 7 of these are also proposed to function in BER (Supplementary Table S2). The major BER enzyme for nuclear DNA is Pol β , while Pol γ is involved in BER of mitochondrial DNA. Moreover, Pols δ and ϵ have been identified in long-patch BER and Pols ι , λ and θ were described to contain AP lyase activities, suggesting a function in BER (reviewed in 37). Indeed, Pol λ is involved in the MUTYH/Pol λ BER sub-pathway [see below: Controlling BER mechanisms by posttranslational modifications (PTMs): future challenges]. The combination of seven Pols with potential functions in BER and the fact that

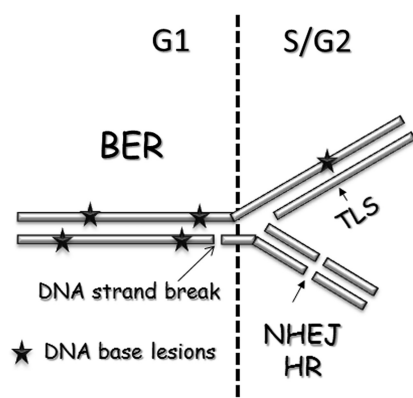


Figure 2. Base excision repair backup involves translesion synthesis and DSB repair pathways. BER is mainly accomplished in the G1 phase of the cell cycle and is also supported by other DNA repair pathways through the cell cycle. A small proportion of DNA base lesions, those which are left unrepaired or generated just before the initiation of replication, are tolerated by TLS. Repair of DSBs arising owing to replication over unrepaired SSBs is accomplished by NHEJ or HR.

11 Pols can perform TLS guarantee reliable backup to BER for the maintenance of efficient and accurate repair of DNA base lesions. This conclusion is supported by the observation that all DNA glycosylase knockout mice (with exception of thymine-DNA glycosylase) are viable and fertile (38), even though they accumulate unrepaired DNA base lesions during their life time, suggesting that the ‘base correction’ function of BER is strongly backed up by TLS (39). However, SSBs unrepaired by BER have the potential to hit the DNA replication fork and to generate DNA double-strand breaks (DSBs) (40), which require either non-homologous end joining (NHEJ) or homologous recombination (HR) for repair (Figure 2). The question is how much backup repair capacity can NHEJ and HR provide to preserve genome stability? Probably not that much because all attempts to generate mice deficient in Pol β , DNA ligase III α or XRCC1, involved in the repair of SSBs, resulted in early embryonic lethality (41–43). Even haploinsufficiency (inactivation of one gene allele) in the Pol β gene leads to significant genome instability and sensitivity to DNA damage, suggesting that BER is the key cellular system responsible for the repair of SSBs (44).

COORDINATION OF BASE EXCISION REPAIR

There are at least two major mechanisms for the coordination of BER reactions that have been extensively discussed in the literature. One mechanism is based on transient protein–protein interactions, while the other suggests preexisting stable repair complexes. The idea that the coordination of the DNA repair process is initiated at early stages was proposed by several groups (45–47). Multiple interactions between BER proteins demonstrated by co-immunoprecipitation, GST-pull downs and a yeast two-hybrid system inspired the ‘passing the baton’ model of BER, which suggests that the repair intermediates of the BER pathway are passed on from one protein to the next in a coordinated manner (48,49). Based on this hypothesis, a

damaged DNA base would be passed during the course of repair from a DNA glycosylase, to APE1, to Pol β , and finally to the XRCC1–DNA ligase III α complex. The ‘passing the baton’ model provides a well-balanced mechanism for the coordination of the ‘classic’ short-patch BER pathway involved in, for example, the repair of uracil in DNA. However, this model does not properly describe the repair of many other DNA base lesions. Even for the repair of oxidative base lesions, it would be difficult to explain how and why a smooth chain of reactions is changed, as the ‘baton’ would need to be passed to one of the DNA damage end-processors.

Several early models also suggested that BER is a continuous process that is performed from the beginning to the end by preassembled DNA repair complexes (45,47). This idea was based on a number of co-immunoprecipitation experiments demonstrating numerous interactions between BER proteins and suggesting that they function in multiprotein complexes [reviewed in (46)]. However, direct attempts to purify repair complexes that are stable in physiological conditions were unsuccessful (50). Because the same subset of BER enzymes (including 11 DNA glycosylases, AP endonuclease, 5 end-processors, 7 Pols and 2 DNA ligases) is involved in the repair of a variety of DNA lesions including damaged DNA bases, AP sites and SSBs of a different nature, it is difficult to imagine that the repair process will be accomplished by a few preexisting DNA repair complexes. Such a variety of different DNA lesions require a DNA repair response tailored to a specific type of DNA damage. Thus, it is reasonable to assume that DNA glycosylases, independent from the rest of BER proteins, are persistently performing high-speed scanning of DNA, removing damaged DNA bases and creating AP sites without nucleation of the DNA repair complexes. Indeed, recent studies on the mechanisms of DNA base recognition and excision by DNA glycosylases support this idea (51,52). Because BER is not the only source of AP sites and a significant proportion of AP sites arises as a result of spontaneous loss of DNA bases, it is also reasonable to conclude that APE1 operates independently from the rest of BER proteins in AP site incision. However, most probably, further repair of SSBs is coordinated by specific protein–protein interactions. This should be initiated by the DNA damage-specific end-processor proteins, all of which are strongly interacting either with Pol β or XRCC1–Ligase III α (4,46) to allow formation of the DNA damage-specific complexes on DNA. As a result, all of these complexes will have a Pol β and XRCC1–DNA ligase III α component, in addition to the DNA damage-specific protein. Indeed, formation of such specific complexes was demonstrated for BER in whole cell extracts by protein formaldehyde crosslinking during repair of SSBs (53).

REGULATION OF SSB REPAIR CAPACITY AND PREVENTION OF DNA DOUBLE-STRAND BREAKS

To survive the challenge of environmental or physiological stress, living systems require the ability to modulate the capacity of BER in response to an increased level of DNA

damage. Most importantly, they should be able to efficiently recognize and repair SSBs to avoid massive formation of DSBs that may overload the cellular DSB repair capacity and eventually lead to cell death. Although mammalian cells have limited amounts of BER enzymes, they are able to recover from acute DNA damage that is significantly above the 'physiological' level. This suggests that mechanisms for instant modulation of BER capacity exist. It has been known for some time that Poly(ADP-ribose) Polymerase 1 (PARP1) molecules bind to SSBs within a few seconds, which activates synthesis of poly(ADP-ribose) polymers and subsequently allows PARP1 to dissociate from DNA (54). Two major models have been proposed to link this PARP1 activity to the BER pathway. First, several groups suggested that poly(ADP-ribosyl)ated PARP1 may recruit BER proteins directly to the DNA damage site, which would impact the DNA repair capacity by providing efficient recognition of SSBs (55,56). However, the results of the experiments testing the role of PARP1 in BER efficiency are contradictory, with some groups finding reduced repair activity in PARP1 depleted cell extracts, while others do not [reviewed in (57)]. One of the earliest models for the role of PARP1 in BER was proposed by Lindahl's group (58). Because their results did not support the idea that PARP1 is required for DNA damage processing, they proposed that PARP1 is involved in protecting DNA SSBs from deterioration by cellular nucleases. Later, Dianov's group also found that although a deficiency of PARP1 does not affect the efficiency of BER reactions (59) and the recruitment of key BER enzymes to sites of DNA damage (60), PARP1 indeed protects DNA SSBs from cellular nucleases (61). Interestingly, PARP1 knockout mice are hypersensitive to alkylating agents and irradiation (62,63). The fact that PARP1 knockout mice develop normally but are sensitive to mutagens suggests that their repair capacity is barely efficient enough to deal with endogenous DNA lesions, but not sufficient to deal with an increased load of DNA damage. It was later proposed (57) that if the molar amount of DNA SSBs exceeds the molar amount of BER enzymes required for repair, PARP1 dimers bind and protect these SSBs from deterioration into more lethal lesions, such as DSBs. Subsequently, PARP1 auto-modification and accumulation of a negatively charged poly(ADP-ribose) chains causes its dissociation from the DNA, allowing BER proteins that are released from the first round of repair to access the SSB to undergo next round of DNA repair (Figure 3). This cycle is repeated whereby PARP1 molecules cycle on and off the DNA and protect the SSBs until repair is accomplished. Because PARP1 is an abundant cellular protein, this mechanism assures an increase in the repair capacity of the cell, thus preventing formation of more deleterious DSBs.

REGULATORY STRATEGIES IN BASE EXCISION REPAIR: THE GOAL IS TO FIT THE NEED

Individual and tissue variations in BER gene expression are significant (64), suggesting that up and down

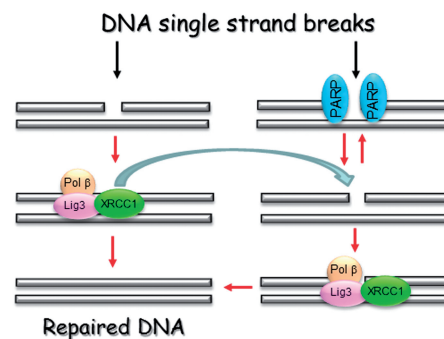


Figure 3. Model explaining the role of PARP1 in the modulation of BER capacity. PARP1 binds and protects SSBs that cannot be repaired immediately owing to excessive SSBs and repair enzyme limitation (Pol β, DNA ligase IIIα–XRCC1 complex) (right branch). PARP1 is activated on binding to SSB and its autopoly(ADP ribosylation) leads to its release from the DNA. This allows BER proteins that are released from the previous round of repair (left branch) to access the SSB and complete the repair process. If unrepaired SSBs remain, PARP1 can cycle on and off the DNA and protect the SSBs until sufficient repair proteins are available. This mechanism increases the repair capacity of BER and prevents the formation of more deleterious DNA DSBs.

regulation of BER is taking place in response to the cellular environment. Because BER is primarily and continuously required by mammalian cells for the repair of endogenously generated lesions, BER activity is regulated to a steady-state level rather than through a mechanism that switches the pathway on and off. To support the error-free gene transcription and replication, steady-state levels of BER enzymes should secure efficient and timely repair of fluctuating amounts of endogenous DNA lesions specific to a particular cell type, or those arising under certain persistent conditions such as hypothermia, hypoxia and inflammation. Indeed, mutations affecting the amounts or enzymatic activities of BER proteins increase genome instability and reduce cell viability (65–67). On the other hand, the amount of BER enzymes should be tightly controlled because their overproduction may affect other DNA transactions and also lead to genome instability and cancer (68–71). To support an adequate level of BER enzymes, cells use an elegant mechanism that links the steady-state levels of BER enzymes to the levels of endogenous DNA damage. This is achieved by stabilization of the key BER enzymes (Pol β, and XRCC1–DNA ligase IIIα) that are conducting DNA repair, and proteasomal degradation of excessive proteins that are not involved in DNA repair. It was recently demonstrated that degradation of excessive BER proteins is supported by two E3 ubiquitin ligases. First, Mule/ARF-BP1 monoubiquitylates unwanted BER proteins and, consecutively, CHIP extends the ubiquitin chain and thus labels proteins for proteasomal degradation (72,73). The control of Mule activity is accomplished by the acute rheumatic fever (ARF) protein, which accumulates in response to DNA damage (74,75). ARF binds to and inhibits Mule activity (76), thus reducing the rate of Mule-dependent ubiquitylation and CHIP-promoted degradation of BER enzymes. The concomitant accumulation of BER enzyme levels leads to

increased DNA damage repair. This in turn results in a reduced level of DNA lesions, reduced release of ARF, activation of Mule and ubiquitylation-dependent degradation of BER enzymes (Pols β and λ (73,77)), thus completing a whole cycle of DNA damage signaling and modulation of BER proteins required for DNA repair (Figure 4). Theoretically, the cellular pool of BER enzymes should include several components: (i) newly synthesized proteins located in the cytoplasm, (ii) enzymes relocated to the nucleus but not yet associated with chromatin and (iii) chromatin-associated proteins involved in DNA repair. The dynamics of this pool are controlled by the cytoplasmic protein Mule, and the nuclear protein ARF that acts as a messenger reporting on the state of DNA repair and controlling Mule activity. Correspondingly, the steady-state levels of BER enzymes are determined by a dynamic equilibrium of all these processes (72,73).

ARF LINKS DNA DAMAGE SIGNALING, REPAIR AND REPLICATION

Although the exact mechanism of ARF induction by DNA damage is still unclear, recent studies support the idea that ARF is a DNA damage reporter (74,75). As we discussed above, ARF interacts with Mule, inhibits

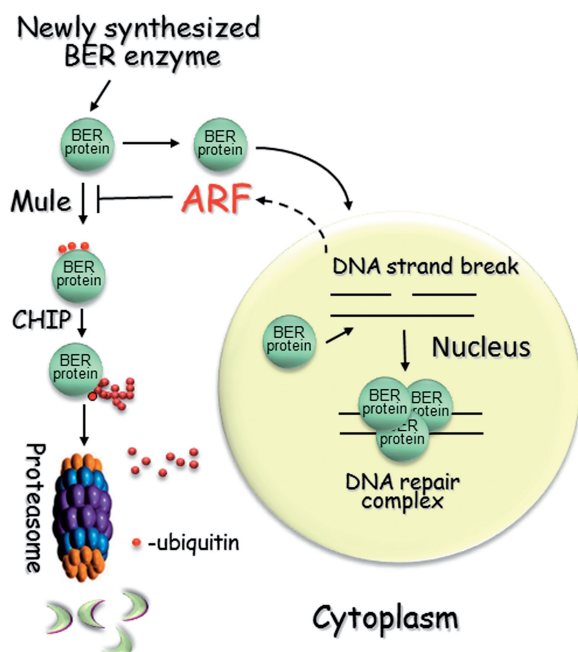


Figure 4. Regulation of steady-state levels of BER enzymes by Mule, CHIP E3 ligases and ARF. Newly synthesized BER proteins are either transported to the nucleus to take part in DNA repair or, if not required for DNA repair, they are ubiquitinated by Mule and then targeted for proteasomal degradation after CHIP-mediated polyubiquitylation. However, following detection of DNA damage, ARF is accumulated and inhibits the activity of Mule, thus reducing BER protein degradation and up regulating nuclear levels of BER enzymes, which elevates DNA repair. Consequently, the repair of DNA damage will result in a decreased release of ARF and a concomitantly increased activity of Mule that down regulates BER protein levels. A new adjustment cycle will therefore begin on the detection of increased levels of DNA damage. Adapted from ref. 73.

its activity and thus up regulates the flow of BER enzymes into the nucleus to support efficient DNA repair (Figure 4). Indeed, it was shown that ARF knockdown by siRNA reduces the rate of DNA repair, while Mule deficiency stimulates it (73). However, it was also demonstrated that ARF induction delays cell cycle progression through the inhibition of the two E3 ubiquitin ligases Mule and Mdm2, which promote p53 ubiquitylation and proteasomal degradation in the absence of DNA damage (76). Taken together, these data indicate that ARF links DNA damage repair and DNA replication. On DNA damage, ARF is induced and thus enhances BER activity through inhibition of Mule and simultaneously, by licensing p53 accumulation, delays DNA replication and cell cycle progression to allow more time for the cell to accomplish DNA repair (Figure 5).

CONTROLLING BER MECHANISMS BY POSTTRANSLATIONAL MODIFICATIONS: FUTURE CHALLENGES

It is evident that the most relevant and elegant way to regulate BER proteins is through various PTMs. These can influence BER proteins at different levels: (i) at the activity level, (ii), at the protein stability level, (iii) at the protein-protein interaction level, (iv) at the cellular localization level, (v) at the transcriptional level and (vi) at the chromatin level. The main PTMs in the regulation of BER proteins identified to date include phosphorylation, acetylation, ubiquitination, SUMOylation and methylation (Supplementary Table S1 and references therein). Although exciting, at the moment this is still an emerging area with many interesting, but disconnected, observations that have not yet been integrated into a comprehensive picture of BER regulation. Nevertheless, some interesting crosstalks between different BER PTMs have been discovered.

As an example for such a crosstalk between two PTMs, we describe the data from our two laboratories on the

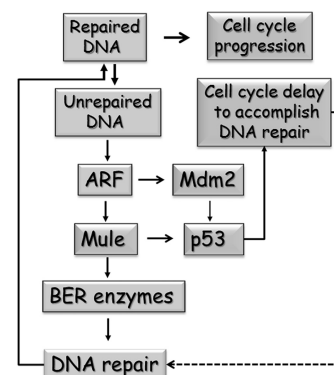


Figure 5. BER is a part of the p53-ARF network controlling genetic stability. BER activity and DNA replication delay are regulated by the same proteins. Detection of DNA damage results in the accumulation of ARF, which activates two cellular processes. By inhibiting Mule, it stabilizes BER proteins and activates DNA repair. At the same time, inhibition of Mule and Mdm2 by ARF leads to an accumulation of p53 and results in a cell cycle delay. After DNA repair is accomplished, the reduction in DNA damage initiates a reverse cycle by reducing DNA repair and releasing the cell for replication.

regulation of Pol λ by phosphorylation and ubiquitylation. The misincorporation of adenosine monophosphate (A) by the replicative Pols α , δ and ϵ opposite to 8-oxo-G is removed by a specific DNA glycosylase called MUTYH, leaving the 8-oxo-G lesion on the DNA. Subsequent incorporation of C opposite 8-oxo-G in the resulting gapped DNA is essential for the further removal of the 8-oxo-G by BER to prevent G-C to T-A transversion mutations (78). In the presence of RP-A and PCNA, Pol λ incorporates a correct C 1200-fold more efficiently than Pol β (79) and is thus important for this branch of BER. Because Pol λ is mainly required for post replication DNA repair, it was reasonable to assume that its expression is coordinated with the cell cycle. Indeed, the cyclin-dependent kinase Cdk2 was identified, in a proteomic approach, as a novel interaction partner of Pol λ (80) and was later found to phosphorylate Pol λ *in vitro*. It was also found that the Pol λ phosphorylation pattern during cell cycle progression mimics the modulation of the Cdk2/cyclin A activity profile. Phosphorylation of threonine-553 is critical for maintaining Pol λ stability, as dephosphorylated protein is targeted to the proteasomal degradation pathway via ubiquitylation by E3 ligase Mule (81). In particular, Pol λ is phosphorylated and stabilized during cell cycle progression in late S and G2 phase, exactly at the point when Pol λ -dependent repair should occur.

CONCLUSIONS

It is conceivable that BER proteins have to be tightly controlled depending on the physiological, and even pathological, situation of a cell. Although we are just beginning to understand how the essential BER pathways and its many involved factors are regulated, BER emerges as the major repair system maintaining genome stability over a lifespan. A complete lack of BER is incompatible with life and a misregulation of BER has been implicated in cancer, neuro-pathology, aging and several other human diseases.

Finally, BER is not an isolated pathway but should be considered as a part of an intricately regulated system that identifies DNA damage, controls DNA repair and coordinates the entire process with cell cycle progression to prevent replication of damaged DNA, and thus guards genome stability. This is achieved by a sophisticated regulatory network that is orchestrated by multiple PTMs, which in turn regulate gene expression, protein stability and interactions of cellular proteins.

Although the entire picture of BER regulation is not yet clear, it is evident that most BER proteins are subject to at least one PTM contributing to the regulatory mechanism. It is also clear that a more definitive picture of cellular BER regulation will be obtained once the opposing reaction enzymes (phosphatases, deubiquinating enzyme, deacetylases and demethylases) are identified.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online: Supplementary Tables 1–2.

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As the authors of this review did not try to assess all current data and opinions on the mechanisms and regulation of BER, but rather tried to be provocative and inspiring, we apologize to many of our colleagues whose important contribution to the BER field was not mentioned. The authors thank Jason Parsons, Keith Caldecott and Florian Freimoser for critically reading the manuscript and for their suggestions.

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Conflict of interest statement. None declared.

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